3D Cancer Cell Culture with Controlled Oxygenation using Bioreactor and Microfabricated Pillars

Michelle A Rose[1,2], Nicole Y Morgan[2], Robert Robey[3], Thomas J Pohida[4], Taylor Sullivan[2], and Michael M Gottesman[3]

[1]Boston University, Boston, MA 02215, [2]National Institute of Biomedical Imaging and Bioengineering, [3]National Cancer Institute, [4]Center for Information Technology, National Institutes of Health, Bethesda, Maryland 20892

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Introduction

Cells grown in a 2D monolayer do not mimic *in vivo* growth. A model of how cells grow in the body would enable better studies of complex diseases and treatment responses. The aim of this project is to grow cancer cell lines in 3D to create a model that more closely mimics *in vivo* behavior of tumors. There are three main components to being able to create such a model:

1. Controlled oxygenation
   - An oxygen gradient can be set up using a two-chambered bioreactor. The bottom chamber has 3% O_2 and the top chamber is anoxic (Figure 1,6). When cells are placed between these chambers, they experience a flow of oxygen from the hypoxic source to the anoxic one, much like cells would *in vivo*.
2. Synthetic vessels
   - The body's vasculature is how cells receive O_2 and other nutrients. Current spheroid culture has reversed O_2 and molecular gradients than in vascularized tumors (Figure 2) [1,2]. Pillars therefore must be fabricated to act as the vessels for spheroids to grow around.
3. Cell-cell and cell-ECM interactions
   - Culturing the cells in Matrigel, an extracellular matrix (ECM), allows the cells to grow while interacting with other cells and an ECM, as actually happens *in vivo*.

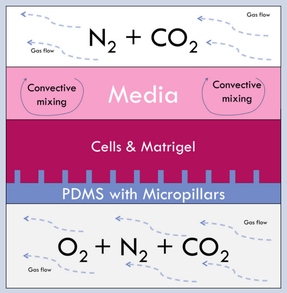

Figure 1. General schematic of bioreactor.

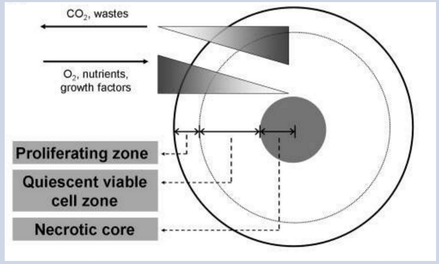

Figure 2. Spheroid with necrotic core [2]. Molecular gradients shown are reversed in vascularized tumors.

Membrane Fabrication

- Cells embedded in 3mg/mL Matrigel and cultured onto PDMS membrane with micropillars on surface (Figure 3,4)
- Polydimethylsiloxane (PDMS) used for its high O_2 permeability
- Membrane fabricated via 3-step process involving photolithography (Figure 3) [1]
- Microstructured PDMS membrane clamped between magnetized acrylic pieces (Figure 4)

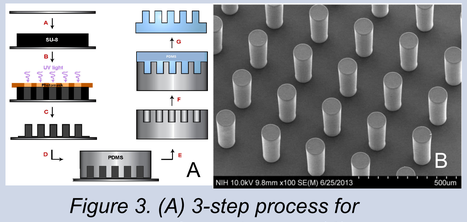

Figure 3. (A) 3-step process for membrane fabrication and (B) scanning electron micrograph of a completed membrane [1].

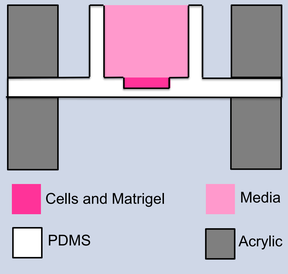

Figure 4. Sandwich layout with cells, PDMS, and acrylic.

Initial Cell Distribution Experiments

- Verify that current protocol optimizes 3D cell growth
- OVCAR8 line transfected with dsRed2 for fluorescence under microscope
- Imaging occurred immediately (1-2 hours) after cells plated
- Different plating conditions tested to see differences in settling (Figure 5)
- MATLAB code developed to approximately quantify amount of settling (Figure 5)

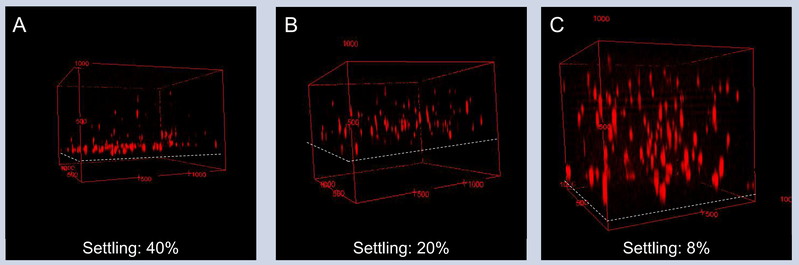

Figure 5. 3D confocal images of cell distributions from free wells under different conditions; (A) no flip upon insertion into 37°C, (B) only flip upon insertion into 37°C, and (C) continuous flipping every 5 minutes. Scales in µm. Dashed lines indicate bottom of PDMS membrane.

Cell Growth Experiments

- OVCAR8-dsRed2 cells (6x10^{5}cells/mL) grown for 7 days in bioreactor (gradient O_2, Figure 6A), hypoxic chamber (3% O_2, Figure 6B), or in 37°C incubator (21% O_2).
- Spheroid density depends on oxygenation (Figure 7)
- Growth surrounding pillars observed (Figure 8)

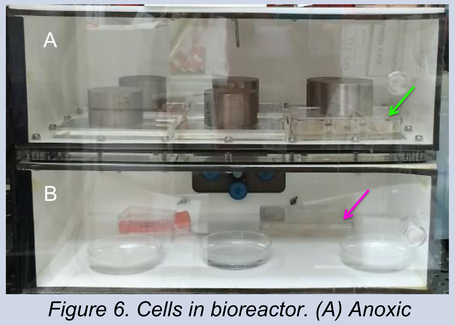

Figure 6. Cells in bioreactor. (A) Anoxic chamber with gradient plate (green arrow). (B) Hypoxic chamber with 3% control plate (pink arrow).

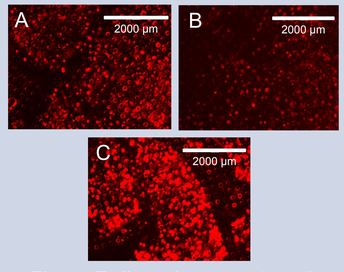

Figure 7. Growth comparison for cells in (A) bioreactor, (B) 3% O_2, and (C) 21% O_2 for 7 days.

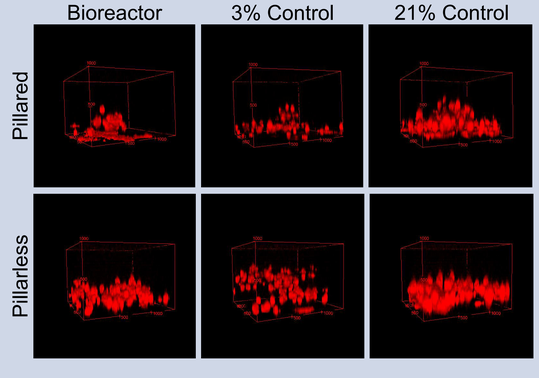

Figure 8. 3D projections from confocal microscope for various conditions of OVCAR8-dsRed2 line grown over 7 days. Scales in µm.

Gene Expression Analysis

- After 7 days of cell growth, two wells per condition harvested for RNA extraction
- TaqMan Low Density Array (TLDA) performed on extracted RNA to quantify different levels of gene expression per condition
- Visual analysis using Partek Genomics Suite (Figure 9)

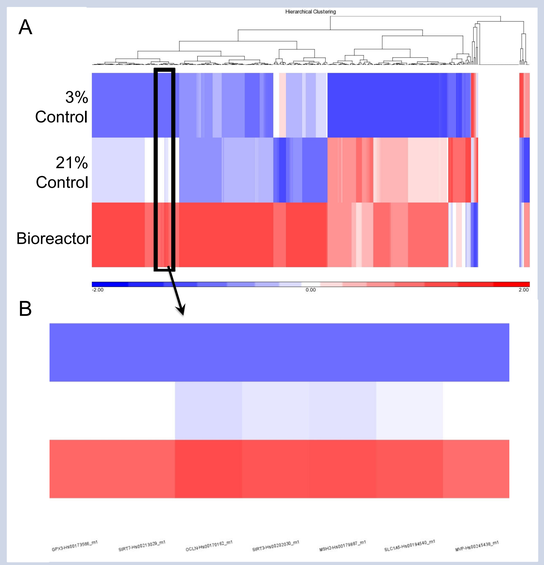

Figure 9. (A) Heat map of gene expression differences between 3% O_2, 21% O_2, and bioreactor. Scale bar indicates number of standard deviations from mean. (B) Close-up of indicated region to show sampling of 7 genes. Note: images shown are from preliminary 3-day cell growth study.

Conclusions and Future Directions

- Cells successfully grown in Matrigel and bioreactor
- Preliminary experiments show differences in cell growth and gene expression based on oxygenation
  - Further confirmation needed from more iterations and in-depth study of gene expression analysis
- Completion and analysis of OVCAR8-dsRed2 7 and 14-day experiment
- Drug penetration and multidrug resistance experiments
- Cell growth experiments with other cell lines
  - MCF-7 (breast cancer) line currently underway
- Extracellular matrices other than Matrigel

References

[1] Jaeger, A.A., et al. Biomaterials. 34(33) p. 8301-13

[2] Lin, R.Z., et al. *J Biotechnol.* 2008. 3(9-10). p. 1172-84

Acknowledgements

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